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and Ethnicity as Risk Factors for Osteoporosis

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FOREWORD

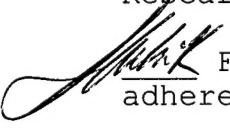
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I. Introduction

This four-year study is a very cost-efficient and timely longitudinal study of bone turnover markers in mid-aged women as they experience the menopause transition. Building on the multisite Study of Women Across the Nation (SWAN), already funded by the National Institutes of Aging and Nursing Research at the National Institutes of Health, this study proposes to analyze already collected and stored specimens of serum to measure bone formation (using an immunoradiometric assay of osteocalcin) and stored urine specimens to measure bone resorption (using urinary N-telopeptide of type I collagen). These two measures will be combined with data from SWAN on bone density (spine, hip and total body), ovarian aging (endogenous sex hormones and menstrual bleeding), medications, medical history, social and psychological assessments, and life style factors (exercise, diet, smoking, body mass) to address four research aims. For each of the aims, specific hypotheses will be investigated using data collected at up to four annual visits as well as menstrual bleeding data collected continually from monthly calendars kept by the subjects. The aims and hypotheses are as follows:

AIM I: To evaluate the relationships between markers of bone turnover (osteocalcin and Type I collagen N-telopeptides) and markers of ovarian aging (reproductive hormone levels, menstrual bleeding patterns, symptoms).

Hypotheses

- I.1 Higher baseline levels of and/or higher rates of change in bone turnover markers will be associated with a higher probability of transition to peri- or post-menopausal status in the 2-year study period.
- I.2 Higher baseline levels of and/or higher rates of change in bone turnover markers will be associated with a higher rate of self-reported peri-menopausal symptoms.
- I.3 The association of bone turnover baseline levels and/or changes over time with the probability of transition to peri- or post-menopause will vary with chronological age (for example, a 50 year old woman may have higher baseline turnover levels and lower rates of turnover change, while a 45 year old woman may have low baseline turnover levels and higher rates of turnover change associated with the same transition probabilities).

AIM II: To determine if one-time (baseline) measures of bone turnover markers or changes over time in these measures are associated with the rate of bone loss over a similar time period.

Hypotheses

- II.1 Elevated levels of baseline bone turnover markers will be associated with a greater bone loss in the subsequent two-year period.
- II.2 An increase in levels of bone turnover markers (measured by at least two points in a two-year period) will be more strongly associated with greater bone loss over the same period than a single, baseline measure of bone turnover.

AIM III: To assess the degree to which potential lifestyle risk factors for osteoporosis (diet, cigarette smoking, exercise, weight) modify the relationships between bone turnover and ovarian aging (Aim I above) and between bone turnover and bone density (Aim II above).

Hypotheses

- III.1 Compared with non-smokers, smokers will have higher baseline levels of bone turnover markers and stronger associations between bone turnover markers and ovarian aging, and between bone turnover markers and bone loss.
- III.2 Women with diets rich in phytoestrogens will have lower levels of bone turnover markers, and weaker associations between bone turnover markers and ovarian aging, and between bone turnover markers and bone loss.
- III.3 Body weight will be inversely associated with bone turnover levels.
- III.4 Heavier women with low levels of physical activity will have weaker associations between bone turnover markers and ovarian aging, and between bone turnover markers and bone loss.

AIM IV: To determine whether the nature or magnitude of the relationships between bone turnover and ovarian aging markers (Aim I above) and bone density (Aim II above) vary according to racial or ethnic

grouping, and whether racial/ethnic differences in lifestyle factors account for any differences with respect to bone turnover markers.

Hypotheses

IV.1 Compared to Caucasians, African American women will have higher levels of bone turnover at baseline. Asian (and Mexican American) women will have baseline bone turnover levels between those for Caucasian and African American women.

IV.2 Racial/ethnic differences in lifestyle factors – such as higher smoking rates among Caucasians and African Americans, greater dietary phytoestrogens among Asians, higher weight among African Americans (and Mexican Americans) – will account for much of the hypothesized differences in baseline levels of bone turnover markers.

IV.3 There will be no detectable differences among different racial/ethnic groups with respect to change over time in bone turnover marker levels.

IV.4 The relationships between bone turnover markers and bone density, and between bone turnover markers and ovarian aging, will vary across racial/ethnic groups, but these differences will be explained in large part by racial/ethnic differences in lifestyle factors and in baseline levels of bone turnover markers.

II. Body

A. Study Objectives

This four-year project seeks to:

- A. Measure osteocalcin (from serum) and Type I collagen N-telopeptides (from urine) using specimens collected annually at three time points (over two years) from 2,250 women at five Field Sites across the U.S. (and, through a separate pending application, from 400 additional women to be recruited at two of the Field Sites); and
- B. Combine these data with pertinent data being collected concurrently on the same women as part of the recently funded SWAN to address the Research Questions and Hypotheses as delineated in Section B above. The results of analyses will be appropriately presented and disseminated.

In order to accomplish the two technical objectives, the following key tasks and timelines were identified at the time of the application:

<u>TECHNICAL OBJECTIVE A:</u>		Measurement and QA/QC of Osteocalcin and Type I collagen N-Telopeptides
Task 1:	Months 1-2:	Finalization of data acquisition protocol
Task 2:	Months 1-2:	Finalization of data forms/electronic file formatting
Task 3:	Month 3:	Finalization of Manual of Operations
Task 4:	Months 2-3:	Design/Testing and implementation of DMS
Task 5:	Months 4-39:	Monthly shipments of specimens to the Central Laboratory
Task 6:	Months 4-38:	Monthly transfer of data results from the Central Laboratory to the Coordinating Center
Task 7:	Months 5-39:	On-going monitoring of Laboratory performance, including site visits

Task 8: Months 6-42: Assessment of the stability of stored specimens using pooled samples

TECHNICAL OBJECTIVE B: Integration of bone turnovers with SWAN data, analyses and results dissemination

Task 9: Months 15-40: Integration go study and SWAN data into analytic data sets as baseline and follow-up annual data become available

Task 10: Months 18-47: Completion of all analyses.

Task 11: Months 18-47: Dissemination of results.

B. Study Progress

Overall, the study has progressed well. The recruitment of participants into the study, particularly the ethnic minorities, occurred at a slower pace and at a greater expense to the parent study than was originally anticipated. Therefore, timelines for the technical objectives have been adjusted to accommodate these unforeseen changes.

1. Participant Recruitment

Recruitment for the study cohort was completed on August 31, 1997. Data collection on those participants recruited toward the end of the recruitment period are scheduled to be completed by December 15, 1997. All recruitment goals for the study were met. Therefore, there are a total of 2,150 participants at the five sites currently participating in the bone densitometry and bone marker study.

2. Completion of Tasks

Tasks identified for Technical Objective B will begin during the second year of funding. Progress on Technical Objective A are detailed below:

TECHNICAL OBJECTIVE A: Measurement and QA/QC of Osteocalcin and Type I collagen N-Telopeptides

Task 1: Months 1-2: Finalization of data acquisition protocol

This task was completed during the first two months of the study. SWAN now has a stable data acquisition protocol. Please see Appendix A for a copy of the specimen collection protocol as related to this study.

Task 2: Months 1-2: Finalization of data forms/electronic file formatting

This task was completed during the first two months of the study. Please see Appendix B for a copy of the specimen collection forms.

Task 3: Month 3: Finalization of Manual of Operations

The manual of operations was completed during the specified time period and is fully operational in the field.

Task 4: Months 2-3: Design/Testing and implementation of DMS

The Data Management System is fully operational for the study.

Task 5: Months 4-39: Monthly shipments of specimens to Central Laboratory

Shipments of specimens are occurring on a monthly basis. The laboratory identified to conduct the bone marker assays is a separate laboratory from the Central Endocrine Laboratory. Because of the slow rate of recruitment and in order to maximize efficiency, the study elected to ship all baseline specimens designated for the bone marker study in mass at the close of baseline data collection. All follow-up specimens are shipped directly to the bone marker laboratory. Therefore, all baseline specimens for the bone marker study will be shipped from the endocrine laboratory to the bone marker laboratory in January, 1998. At that time, assays will be run in batch, with results for baseline and follow-up 01 available shortly afterward.

Task 6: Months 4-38: Monthly transfer of data results from Central Laboratory to the Coordinating Center

Once analysis of bone marker assays begins, data will be transferred from the bone marker laboratory to the Coordinating Center on a monthly basis. This procedure is tested and fully operational.

Task 7: Months 5-39: On-going monitoring of Laboratory performance, including site visits

The bone marker laboratory was site visited in July, 1996 and found to be operating properly with all procedures being conducted as required.

Task 8: Months 6-42: Assessment of the stability of stored specimens using pooled samples

The SWAN study's Laboratory Oversight Committee is charged with regular review of the laboratory's Standard Operating Procedures and QC data. The LOC has reviewed all SOPs and QC data for assays associated with this study and have found them to meet or exceed all standards.

Conclusions

There are no research conclusions to report at this time. Baseline and Follow-up 01 study results will be available in the next annual report.

References

Not Applicable

Appendices

Appendix A**SWAN Manual of Operations, Laboratory Protocol**

6.1 OVERVIEW OF SWAN LABORATORIES

6.1.1 Samples and Laboratories

The Study of Women's Health Across the Nation (SWAN) will include the collection of biological samples from women at seven Clinical Centers. These samples will be shipped to participating laboratories with desired capabilities. The designated laboratories will examine the samples for analytes agreed to by the SWAN Steering Committee using procedures to ensure comparability of results over the many years of this longitudinal study. These procedures involve inclusion in every assay of carefully preserved, quality control samples that are available in amounts sufficient to last up to nine years. Upon completion of an analysis (which may include repeat determinations), residual samples will be archived and results will be transferred electronically to the Coordinating Center where they will be edited and then released to the participating Clinical Centers.

The two laboratories selected to date are the Endocrine Sciences Program (RSP) Central Laboratory at the University of Michigan, where endocrine markers will be measured, and the Medical Research Laboratories (MRL) in Highland Heights, KY where bone markers and cardiovascular markers will be measured. Other laboratories may be used for analysis of other substances. The following describes what is being collected at annual follow-up visits. The remainder of the manual describes details of procedures unique to the RSP laboratory, procedures unique to the MRL laboratory and an integrated summary of the combined operation.

6.1.2 Sample Collection and Processing Overview

Annual samples of blood for measurements of endocrine, cardiovascular and bone markers, and for repository, will be obtained between days two and five of a woman's menstrual cycle.

- Samples should be obtained under fasting conditions (no food or drink in the previous 12 hours, **except water**) preferably between 8:00 a.m. and 10:00 a.m. Subjects should be seated quietly for five minutes immediately prior to the draw.
- A total of 37 mL of blood will be collected (5 Vacutainer):
 - Endocrine measures and repository specimens require collection into one 10 mL red top (serum) tube.
 - Cardiovascular measures and repository specimens will require collection of 27 mL, in sequence, into
 - an additional 10 mL red top (serum) tube;
 - a 5 mL blue top (citrate) tube;
 - a 7 mL purple top (EDTA) tube and;
 - a 5 mL blue top (citrate) tube.

- The Blue Top and Purple Top tubes can be stored in a 4°C refrigerator (or cooler) for up to a maximum of two hours prior to centrifugation. Immediate centrifugation is preferred.
- The Red Top tubes should sit at room temperature from a minimum of 30 to a maximum (preferred) of 60 minutes to allow the clot to form and then be refrigerated (4°C) from a minimum of 30 to a maximum (preferred) of 60 minutes before spinning.
- Serum and plasma will be aliquotted into pre-packaged bar-coded aliquot tubes in accordance with subsequent detailed instructions.
- The phlebotomist should write the Subject's three initials and the date of draw on the label of each tube. The subject ID label should be attached to the MRL Specimen Collection Log and the Specimen Collection Record.
- All aliquotted samples will be frozen at -20° C or below at the site. These specimens must be frozen at least overnight before they are shipped to the respective laboratories on dry ice.
- An early morning (before 9:00 AM) sample of urine for measurement of bone markers and repository will be collected from Subjects at the time of, or immediately prior to, their visit. At the site, the sample will be divided into five aliquots of 1 mL each, frozen at -20° C (or below) and shipped monthly on dry ice as follows:
 - 1 x mL to MRL for bone marker measurements
 - 4 x 1 ml to RSP for repository

6.2 LABORATORY CONTACT INFORMATION

ALL SPECIMEN COLLECTION PACKETS WILL BE PROVIDED BY RSP CENTRAL LABORATORY.

Questions for the University of Michigan Central Laboratory (RSP) should be addressed to:

Address:

RSP CLASS:
1919 Green Rd., Room A120A
University of Michigan
Ann Arbor, MI 48105 - 2554

Contact Person(s):

Holly Anderson-Davis, Lab Manager
Kimberly Gonzalez, Lab Supervisor
313-747-0256 (HAD)
313-763-2461 (KG)
313-936-8620 FAX
HAD@umich.edu
Gonzaleb@umich.edu

IF CALLING TO ORDER ADDITIONAL PACKETS, BE PREPARED TO PROVIDE THE FOLLOWING INFORMATION.

1. Number of Subjects for whom follow-up packets are needed.
2. Address to which the packets should be shipped.
3. Whether routine or emergency delivery is required.

Requests for supplies including shipping labels, shipping forms, shipping containers, dri-mop absorbent, leak-proof jars and Airborne Express airbills, as well as answers to questions. and resolution of problems at Medical Research Laboratories should be directed to:

Address:

Medical Research Laboratories
2 Tesseneer Drive
Highland Heights KY 41076

Contact Person(s):

Michelle Falke
Paula Steiner
Traci Turner
1-800-323-2996
1-606-781-8877
FAX 1-606-781-9310
pmsteiner@aol.com

6.3 SPECIMEN COLLECTION AND PROCESSING

See Figures 6.1 and 6.2 for detailed summaries of specimen processing and shipping. These figures should be copied and posted where specimens are processed.

6.3.1 Specimen Collection Supplies Kit

An instruction set (Figures 6.3a and 6.3b) will be included with each shipment of Kits and should be posted where specimens are processed. Specimen collection supplies from both SWAN laboratories will be combined and provided to study sites in modules of two kits by RSP to make up one packet per subject. Each packet will include all supplies for collection of specimens for:

- Endocrine markers;
- Cardiovascular markers;
- Serum and urine bone markers;
- Repository samples (serum, plasma and urine).

For each follow-up visit requiring samples, RSP will provide the two kits in zippered plastic bags, all in a single zippered packet (Module). The contents of the Module are as follows:

One Urine specimen collection cup

Specimen Collection Log/Specimen Collection Record (Figures 6.4a and 6.4b)

Blood Drawing Kit:

- One prelabeled red top (serum) Vacutainer tube (endocrine measures)
- One unlabeled 10 mL red top (serum) Vacutainer tube (glucose, insulin, bone markers)
- Two unlabeled 5 mL blue top (citrate plasma) Vacutainer tube (clotting factors)
- One unlabeled 7 mL purple top (EDTA plasma) Vacutainer tube (lipids)
- One double-ended Vacutainer needle

Blood Aliquotting Kit:

- Two prelabeled red cap large serum vials (RSP)
- Four prelabeled red cap small serum vials (RSP)
- Two unlabeled red cap large serum vials (MRL)

- One unlabeled blue cap large citrate plasma vial (MRL)
- Four prelabeled blue cap small plasma vials (RSP)

- Three unlabeled green cap large EDTA plasma vials (MRL)

- One unlabeled yellow cap small urine vial (MRL)
- Four prelabeled yellow cap small urine vials (RSP)

- Six transfer pipets (three with markers at 1 mL and 2 mL & three without markers)
- Three absorbent pads

6.3.2 Specimen Labeling/Logging Procedures

Each Study Laboratory has a distinct system for labeling specimens.

Michigan (RSP) pre-labels all Vacutainer and transfer vials using a label containing a unique bar code and a common, highly visible three digit number. These bar-coded Vacutainer and transfer vials are prepared in 2 separate kits by RSP and supplied as a component in the combined specimen collection module.

MRL preprints bar-coded labels (incorporating the SWAN study ID) in advance for MRL specimens required by the protocol (**Figure 6.5**). Multiple labels are provided for each MRL specimen so that there is one label for each Vacutainer tube collected, one for each transfer vial, and one label for the MRL Specimen Shipping Packing List (**Figure 6.6**) that is to accompany each shipment. Extra labels will be provided upon request. MRL labels are affixed at the study site. MRL labels must be obtained directly from MRL and are not included in the specimen collection kits.

The following steps should be followed when preparing for specimen collection.

1. Remove the unlabeled MRL tubes.
2. Locate the correct sheet of MRL labels (**corresponding to the Subject's assigned ID and visit number**) for the Subject from whom the samples will be drawn.
3. Enter the date of the draw and **THREE** Subject initials on all the tube labels, corresponding to the first, middle and last name. Dashes should be substituted if no middle name is given. It is important that the **initial letters be printed clearly** in plain block letters.
4. Place the completed labels onto the unlabeled MRL collection tubes and unlabeled MRL transfer vials taking care to position the **bar code as straight as possible along the length of the tube**. (**Figure 6.7**)
5. It is necessary that the draw date correspond with the date of the visit. Therefore, **DO NOT** enter draw dates ahead of time.

NOTE: All tubes sent to the laboratories with improperly applied or incorrectly completed SWAN ID labels will be returned immediately to the site for correct processing. All shipments will be checked for adequate volumes, correct packaging and labeling, accidental thawing or breakage. The CC and site will be informed of any problems.

6.3.3 Specimen Collection

(a.) Scheduling of Blood Draws

1. For Respondents with regular bleeding (predictable within a week), including those who are currently using prescription HRT, a fasting blood specimen should be obtained between 8 - 10 a.m., during days 2 - 5 of her menstrual cycle window. For Respondents who have had a hysterectomy or no bleed in past 3 months, a fasting blood sample should be drawn between 8 - 10 a.m. within the data collection window.
2. Within the first 60 days of initiation of follow-up data collection, the site will attempt to obtain a fasting blood sample drawn during days 2-5 of the Respondent's menstrual cycle. If the site determines during a blood draw attempt that the Respondent is not fasting or is not in days 2-5 of her menstrual cycle, then the blood sample should not be taken at that time and another attempt made at a later date. Depending on a woman's menstrual regularity, there may be one or more opportunities for a blood draw in the menstrual window during the first 60 days.
3. After 60 days have elapsed, the site will attempt to schedule a respondent's blood draw whenever it is convenient and feasible, without regard to day of menstrual cycle. On this last attempt, sites should try to get a fasting blood sample if at all possible, but if this is not possible the blood still should be drawn.

(b.) Blood Collection

1. Seat the Subject for at least five minutes prior to blood collection. Blood draw is preferably completed between 8:00 a.m. and 10:00 a.m. It should be noted on the Specimen Collection Log what position the Subject is in (i.e., sitting, lying down) and the same position used for drawing blood at baseline and each annual follow-up. This information will be printed on the Respondent's Contact Record. Subjects must be fasting for ALL specimen collections. Fasting is defined as nothing to eat or drink except water for at least 12 hours.
2. Using a Vacutainer, double ended needle, draw the blood in the following order:

Draw #1 10 mLs	Red Top Vacutainer (pre-labeled RSP, repository), immediately invert 8-10 times;
Draw #2 10 mLs	Red Top Vacutainer (MRL, repository), immediately invert 8-10 times;
Draw #3 5 mLs	Blue Top (Citrate) Vacutainer (MRL, repository), immediately invert 8-10 times;
Draw #4 7 mLs	Purple Top (EDTA) Vacutainer (MRL), immediately invert 8-10 times.
Draw #5 5 mLs	Blue Top (Citrate) Vacutainer (repository), immediately invert 8-10 times;

A butterfly needle (21g) and/or 5 mL (pediatric) Vacutainer may be used on women with smaller veins. MRL will provide the sites with a supply of smaller Vacutainer to have on hand should the need arise.

THE SAMPLES MUST BE DRAWN IN THIS ORDER TO PREVENT THE CARRY-OVER OF VACUTAINER ADDITIVES INTO THE SERUM TUBE.

(c.) Urine Collection

At the time of, or immediately prior to, her study visit, the Subject shall be asked to collect an early morning sample of urine. This sample should be collected before 9:00 AM. Remember to record the estimated time of urination. This sample should be refrigerated upon receipt by the site, unless it is aliquotted immediately.

6.3.4 Serum Sample Processing

Gloves must be worn at all times when handling specimens. This includes during the removal of the rubber stopper from the blood tubes, centrifugation, pipetting, disposal of contaminated tubes, and clean up of any spills. Tubes, needles, and pipets must be properly disposed in biohazard containers, in accordance with institutional requirements.

(a) BEFORE CENTRIFUGATION

Red Top Tubes should sit at room temperature from a minimum of 30 to a maximum (preferred) of 60 minutes to allow the clot to form and then be refrigerated (4°C) from a minimum of 30 to a maximum (preferred) of 60 minutes before centrifugation.

Blue and Purple Top Tubes should be centrifuged immediately or placed immediately in a cooler (on blue ice or wet ice) or in a refrigerator (4°C) for up to 2 hours. It is preferred that the blue and purple top tubes be centrifuged immediately.

(b) CENTRIFUGATION

Within 2 hours of collection, all tubes should be centrifuged:

- in a refrigerated centrifuge (4°C)
- at a minimum force of 1300g
- for 20 minutes

(c) ALIUOTTING

- Use plastic, disposable pipets provided in the kits to transfer the serum, changing to a new pipette when you change to a new blood draw tube.
 - * From red top Vacutainer #1, Pipet 2 mL serum to each large vial (S1, S2) and the remaining serum into the smaller vial (S3). Close tightly.
 - * From red top Vacutainer #2, Pipet 1 mL serum to each of the larger vials (S4, S5) and split the remaining serum between the other three (3) smaller vials (S6, S7, S8) in ~1mL aliquots.
 - * From a blue top Vacutainer, transfer 1 mL plasma to the larger MRL blue cap tube (P1) and the remainder of both blue top Vacutainers to the four (4) blue cap RSP vials (P2, P3, P4 P5) in ~1 mL aliquots. Aspirate NO cells.
 - * From the purple top Vacutainer, transfer three (3) 1 mL plasma aliquots to the three (3) larger MRL green cap tubes (E1, E2, E3). Close vials tightly.
- Should cells or platelets be aspirated in the aliquotting process, the tubes should be centrifuged a second time to prevent the carryover of cells or platelets to the sample.
- Carefully match ID labels on the collection tubes with ID labels on corresponding aliquot tubes from the same kit.
- Complete the Specimen Collection Record (Figure 6.4 a.-b.).

- Affix designated labels on the current MRL Shipping Log (Figure 6.6) corresponding to the current shipment being filled, of which the subject's specimens are a part. See shipping instructions, Section 6.5 below.

- Check that ID labels are correctly affixed to the Specimen Collection Record.
- Note any problems such as insufficient volume, spills, etc. on the Specimen Collection Record.

(d) STORAGE

- Check that all aliquot tube caps are secure.
- Check that all labels are secure on the tubes.
- Return all aliquots to the appropriate shipping bags, seal bags securely and place tubes upright in a -20°C (or below) **non-defrosting** freezer. Carefully monitor the freezer temperature. Frozen specimens can be stored locally for no more than 30 days and must be stored at least overnight before shipping. Splitting specimens into the designated shipping bags **prior** to freezing will eliminate the need to open frozen bags later and will avoid partial thawing of specimens when preparing for shipping.

6.3.5 Urine Specimen Processing

- (a) HANDLING: While the urine need not be refrigerated between the time of collection and delivery to the site that same morning, it should be protected from direct sunlight by wrapping in aluminum foil or placing in an envelope. Immediate aliquotting and freezing is preferred. If not aliquotting immediately, clinic personnel should refrigerate (4°C) the urine upon receiving it from the subject or place in a cooler on blue ice for not more than 4 hours.
- (b) ALIUOTTING: Using a plastic pipet, transfer 1 mL quantities into each of one unlabeled (MRL/bone markers) and four prelabeled (RSP/repository) yellow capped vials.
- (c) STORAGE: Close the vials tightly and freeze at -20°C or below until packaged with dry ice for monthly shipment to MRL for bone markers (1 x mL) and RSP for repository (4 x mL). Write in the notes section on the Specimen Collection Record form any comments such as problems with the urine collection, insufficient volume, spills, etc.

6.3.6 Blood Volumes and Priorities

The goal of the collection protocol is to minimize the total blood volume and the study site's burden for aliquotting and shipping while preserving maximum flexibility for assays. Key features are:

- A total blood volume of not more than 37 mL will accommodate all endocrine hormone assays, glucose, insulin, clotting factors and lipids.
- 4 x 1 mL serum samples, 4 x 1 mL plasma samples and 4 x 1 mL urine samples will be stored (**without thaw**) at RSP or another site (to be identified) for future assays (to be determined) as well as other ancillary studies.
- Each Laboratory will also store additional thawed and re-frozen serum after completion of assays.

SWAN ANNUAL FOLLOW-UP

MANUAL OF OPERATIONS

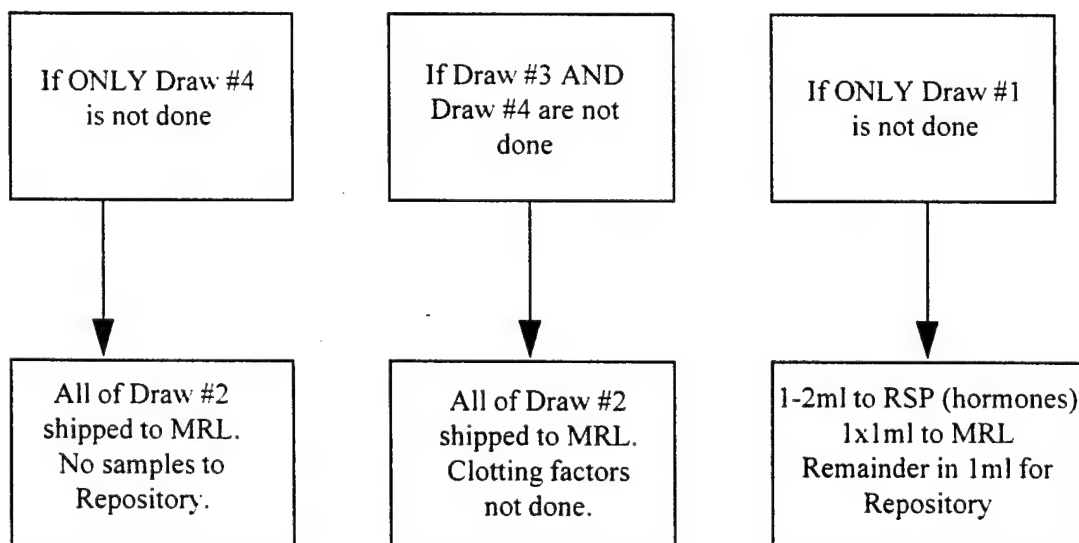
2/10/97

Section 6. Page 9

Refer to the following guidelines if the blood draw results in **insufficient volume**:

1. If Draw #4 (EDTA-Purple top) is not done at all, all of Draw #2 (Second Red top) will be shipped to MRL. No samples from this draw will go to repository. (See NOTE below.)
2. If neither Draw 3 or 4 are collected, all of Draw #2 will be shipped to MRL as above. Clotting factors will not be done. (See NOTE below.)
3. If ONLY Draw #1 is completed, aliquot as follows: 2 mL in 5 mL vial to RSP for hormone assays, 1 mL in 3.5 mL vial to MRL for glucose and insulin and remainder in 1 mL aliquots to RSP for repository.

NOTE: If either Draw #4 (Purple Top) or both Draws #3 (Blue Top) and #4 are not collected, ALL specimens from Draw #2 (Red Top) must be shipped to MRL. In such cases, aliquot as follows: 1 mL into the 3.5 mL Red cap vials provided by MRL and 3 x 1 mL into the 3.5 mL Green cap vials provided by MRL. Write SERUM on the label for the Green cap vials. On the Specimen Collection Record form, indicate in the Notes that the three 1 mL serum repository aliquots (S6-S9) have not been collected and record "0.00" in the volume spaces for these aliquots. Also record in the Notes for the EDTA Plasma aliquots E1-E3, that the tubes contain SERUM, NOT PLASMA because of a short blood draw.



6.4 FORM COMPLETION/PROCESSING

One (double-sided) form is associated with each Subject's specimens:

- Specimen Collection Record (**Figure 6.4 a-b**)

This form must be completed for each set of the Subject's specimens collected under the core protocol. Instructions for completion and data entry are provided below.

6.4.1 Specimen Collection Record

This form must be completed for each subject at the time of phlebotomy, urine collection and specimen processing.

Information on both sides of this form will be entered into the SWAN database. Originals should be kept on file.

In Section A, (Figure 6.4a) affix a Subject ID label (A1) and record the study visit (A2), the date completed (A4), the subject's date of birth (A5), whether the subject was sitting or lying down during the blood draw (A6), whether or not a butterfly was used (A7), and if so, what gauge (A7A).

Section B provides places for each draw or collection to: check whether the blood draw was completed (B1), record the collection date and time (B2 & B3), record the initials of the Phlebotomist (B4), check whether sample was refrigerated before spinning (B5), record the start time of centrifugation ("spin time") (B6), record the time placed in storage and temperature (B7), and record the initials of the Laboratorian (B8).

Any relevant notes about the blood draw or urine collection (e.g. pediatric Vacutainer used, partial draw obtained) should be recorded in Section B at question B9.

NOTE: All specimens should be centrifuged within 2.0 hours of the time they are collected. To facilitate completion of this form, boxes can be checked for dates and times if they are the same for all collection tubes.

Section C (Figure 6.3b) on the second side of this form asks for aliquotting information for every specimen expected (i.e. RSP, Repo and MRL) at the follow-up visit. If all the aliquots for a given Vacutainer tube were obtained, simply check the box for the corresponding Vacutainer (i.e., C1 for the first red top Vacutainer through C6 for the 5 urine aliquots). Record the volumes collected for all aliquots for that Vacutainer, to the nearest 0.25 ml. If an aliquot is not obtained, enter "0.00" in the volume space.

In the bottom center of the second side of this form, record any aliquot specific notes relevant to this draw (i.e. problem with blood draw, insufficient volume, spills) as needed. Each aliquot has been assigned a code, i.e., S1-S3 for serum aliquots from the first RSP red top tube. When recording notes, identify the corresponding aliquot(s) using this code.

The bar codes on the second side of this form are entered into the SWAN DMS by "wandering". If a given bar code cannot be wanded, the associated number can be entered manually or the bar code on the associated tube can be wanded. File the original forms. Do not send any sheets to the RSP or MRL Laboratories. Instead, information on this form will be transferred electronically to RSP from the SWAN DMS.

6.5. SHIPPING OF SPECIMENS

6.5.1 Specimen Shipping Records

Each specimen should be recorded on one of the following forms at the time of processing:

- Specimen Shipment Packing List (Frozen Shipment) (MRL) (**Figure 6.6**)
- Shipping Record (RSP) (**Figure 6.8**)

Specimen Shipment Packing List (Frozen Shipment): Affix one label to the packing list for each vial being shipped. Labels for specimens from multiple study participants may be placed on a single packing list. Multiple packing lists may be included with a shipment. Please keep a photocopy of this packing list(s) for your records and submit the original(s) with the specimens.

Shipping Record to RSP: Complete this form for each shipment to RSP. The information is to identify each bag of samples included in a single shipment. Attach a copy of the designated shipper's airbill to the completed sheet. Optionally attach a printed copy of the e-mail message acknowledging receipt of the samples at RSP. File the attached sheets locally as part of the site records. Do not send this form with the shipment.

Table 6.1
Integrated Summary of Specimen Shipping

Summary Table:

	Purpose	Vol	Medium	Label	Cap	Store	Ship To	Freq	Packing
1	Endo. Hormones	1 x 2 mL	Serum	RSP	Red	<-20C	RSP-1	Monthly	Dry Ice
2	Repository Serum	2 x 1 mL	Serum	RSP	Red	<-20C	RSP-1	Monthly	Dry Ice
3	Repository (Citrate)	2 x 1 mL	Plasma	RSP	Blue	<-20C	RSP-1	Monthly	Dry Ice
4	Urine Repository	2 x 1 mL	Urine	RSP	Yellow	<-20C	RSP-1	Monthly	Dry Ice
5	Glucose; Insulin	1 x 1 mL	Serum	MRL	Red	<-20C	MRL	Monthly	Dry Ice
6	Bone Markers	1 x 1 mL	Serum	MRL	Red	<-20C	MRL	Monthly	Dry Ice
7	CV Markers (Citrate)	1 x 1 mL	Plasma	MRL	Blue	<-20C	MRL	Monthly	Dry Ice
8	Lipids (EDTA)	3 x 1 mL	Plasma	MRL	Green	<-20C	MRL	Monthly	Dry Ice
9	Urine Bone Markers	1 x 1 mL	Urine	RSP	Yellow	<-20C	MRL	Monthly	Dry Ice
10	Endo. Hormones (backup)	1 x 2 mL	Serum	RSP	Red	<-20C	RSP-2	Monthly	Dry Ice
11	Repository Serum	2 x 1 mL	Serum	RSP	Red	<-20C	RSP-2	Monthly	Dry Ice
12	Repository (Citrate)	2 x 1 mL	Plasma	RSP	Blue	<-20C	RSP-2	Monthly	Dry Ice
13	Urine Repository	2 x 1 mL	Urine	RSP	Yellow	<-20C	RSP-2	Monthly	Dry Ice

Instructions:

General: Be sure all tubes and vials are closed securely. Ship Monday through Wednesday only. Ship all vials to their respective laboratory monthly, after residing at least overnight in a freezer at or below -20° C. Ship by Airborne Express, 1-800-247-2676 (to MRL) or other shipper (to RSP). RSP shipments are being split (RSP-1 and RSP-2) as a precaution against shipping loss or mishandling and should be sent in different monthly shipments. Notify the lab via e-mail when a shipment has been sent. RSP will acknowledge receipt of shipment via an e-mail message to the site. MRL will acknowledge receipt of a shipment only if confirmation is requested.

6.5.3 Monthly Shipment to MRL: Seven tubes with MRL labels will be stored and shipped frozen, roughly at monthly intervals:

two **red top tubes** with ~1 mL serum (glucose, insulin and bone markers);
 one **blue top tube** with ~1 mL citrated plasma (cardiovascular markers);
 three **green top tubes** with ~1 mL EDTA plasma (lipids) and
 one **yellow top tube** with ~ 1 mL urine (bone markers)

For shipping, place the collected, sealed bags of frozen tubes destined for MRL into a Styrofoam shipping container with ~5 lbs dry ice. Close the Styrofoam lid securely and place a spacer tray on top of the carton. Make a copy of the MRL Frozen Specimen Shipment Packing List (blue sheet, **Figure 6.6**) and place the original blue Packing List with affixed labels in the top section of the container. Close and seal the outer carton. Attach to the shipping box: the Airborne Express label addressed as above, a Lab Pack sticker, and the red and black biohazard label. Affix a Dry Ice Label (9, International Goods) reading: "Dangerous goods, shipper's declaration not required. Dry Ice 9, UN 1845, 1 x 2.2 kg" and add your name and address as sender. Affix an airbill to the carton and ship by Airborne Express. Retain the shippers copy of the airbill and attach it to the copies of included Specimen Shipment Packing Lists. Ship to:

Medical Research Laboratories
2 Tesseneer Drive
Turner
Highland Heights, KY 41076

Contact:
Michelle Falke, Paula Steiner or Traci
1-800-323-2996; 1-606-781-8877

Ship by Airborne Express (MRL Airborne account number is 32663480). Retain the shipper's copy of the airbill and attach it to the copies of the included Specimen Shipment Packing Lists for the site file.

6.5.4 Monthly Shipment #1 to RSP: Of the remaining 14 frozen vials, seven (RSP-1; Table 6.1) should be sent in one shipment and seven (RSP-2) in a second shipment on a different day, (but RSP-2 vials from different subjects may be included in an RSP-1 shipment). Each shipment should be sent roughly at monthly intervals. The RSP-1 shipment (rows 1-4, Table 6.1) is to include:

one **red cap tube** with ~2 mL Serum (endocrine hormones);
two **red cap vials** with ~1 mL Serum (repository);
two **blue cap vials** with ~1 mL Citrate plasma (repository), and
two **yellow cap vials** with ~1 mL Urine (repository).

This separation of tubes may be accomplished most easily just before freezing by separating each subject's samples into two zippered bags: RSP-1 and RSP-2, each with Dri Mop pads. Assemble all zippered bags awaiting shipment, with only one bag per subject in a shipment, complete the Shipping Record to RSP (**Figure 6.8**), and transfer bags to a corrugated cardboard box (provide locally). Put the box into a reusable Styrofoam shipping container (e.g. Thermosafe, #399 @\$38.35; 800-323-7442). Add approximately 5 lbs of dry ice, close the Styrofoam lid securely and then close and seal the outer container. Identification of included samples will be sent electronically; no paper work should be included with the shipment.

Attach to the shipping box the red and black biohazard label and a shipping label addressed to:

RSP CLASS Laboratory
University of Michigan

1919 Green Road, Room A120A
0256 (HAD)
Ann Arbor, MI 48105-2554

Contact:
Kimberly Gonzalez or Holly Anderson-
Davis
1-313-763-2461(KG) or 1-313-747-

Affix a Dry Ice Label (9, International Goods) reading: "Dangerous goods, shipper's declaration not required. Dry Ice 9, UN 1845, 1 x 2.2 kg" and add your name and address as sender. Ship prepaid (by site) for overnight delivery by the shipper. Attach the shipper's copy of the airbill to the completed RSP Shipping Record RSP and file the forms locally in a safe place. Confirmation of receipt of samples will be sent by e-mail.

6.5.5 Monthly Shipment #2 to RSP: The final seven vials from a single subject, all stored frozen (RSP-2; see Table 6.1), should be put into one bag and sent to RSP roughly at monthly intervals in a separate shipment on a different day than samples from the same subject(s) shipped as RSP-1 (but RSP-2 vials may be included with RSP-1 shipments for other subjects). These vials (rows 10-13, Table 6.1) should include:

one **red cap tube** with ~2 mL Serum (backup for endocrine hormones);
two **red cap vials** with ~1 mL Serum (repository);
two **blue cap vials** with ~1 mL Citrate plasma (repository), and
two **yellow cap vials** with ~1 mL Urine (repository).

Follow the instructions above for RSP-1 vials. Empty Styrofoam shipping boxes will be returned by UPS.

6.5.6 Supplies Furnished by MRL

Shipping Supplies

Insulated Shipping Containers
Hazardous Goods (Dry Ice) Labels
Specimen Shipment Packing Lists (blue)
Airbills for Overnight Courier

Additional Supplies

MRL Specimen Label Sets
5 mL (Pediatric) Red and Blue Top Vacutainer

6.5.7 Supplies to be Purchased by Site for RSP Shipments

Reusable Styrofoam shipping container (e.g. Thermosafe, #399 @\$38.35; to order call 800-323-7442) Dry Ice Label (9, International Goods)

6.6 LABORATORY PROCESSING OF SPECIMENS

6.6.1 Specimen Processing at Michigan (RSP)

- On receipt, vial bar codes are wanded and matched with numbers entered at sites.
- Specimen quality is evaluated and any problems noted in the database.
- Repository (serum, plasma, urine) samples and working (serum) samples are placed into a -80° C freezer and the bar code associated with freezer shelf is also recorded by wanding.
- The resulting electronic recording is uploaded into a sample locator database which serves to establish receipt and storage location of every sample.
- In addition to managing storage locations, the system generates pick lists based on priorities set by users and laboratory personnel, monitors processing and freeze-thaw cycles and generates lists for storing re-frozen samples.
- Results are transferred electronically to the Coordinating Center monthly.
- Insulated shipping boxes are refurbished and returned by UPS second-day service.

6.6.2 Specimen Processing at Medical Research Laboratories

- Specimens are received and entered into a computer log. Test requests are generated automatically.
- Specimen quality is evaluated.
- Lipid profiles are performed within a few hours of receipt. Remaining aliquots will be stored at
- 80° C and freezer locations tracked, for testing at a later date.
- *Planned tests include:* Fibrinogen, Factor VIIc, PAI-1, TPA Antigen, Glucose, Insulin, Lipoprotein Lp(a) and Lp(A1).
- Data are reviewed before release.
- Results are transmitted electronically to the Coordinating Center monthly.
- Insulated shipping boxes are refurbished and returned by second-day service.

6.7 ASSAYS AND QC PROCEDURES PERFORMED AT STUDY LABORATORIES

6.7.1 Assays to be Performed at Michigan (RSP)

Analytes to Be Measured: Current plans call for running combinations of follicle stimulating hormone (FSH), luteinizing hormone (LH), dehydroepiandrosterone sulfate (DHEAS), estradiol, testosterone and sex hormone binding globulin (SHBG) on samples of serum.

6.7.2 Assay and QC Procedures at Michigan (RSP)

Laboratorians will locate samples in accordance with pick lists generated for that day, and rapidly thaw the sample. Immediately upon thawing, the samples and associated QC preparations will be transferred to a carousel and the loaded carousel inserted into one of two automated Ciba Corning Diagnostics ACS:180 analyzers. The assays are run using reagents provided for the ACS:180 or acquired elsewhere and proven to give results comparable to commercial kits for the intended analytes, with special emphasis on specificity and reliability. Proteins are analyzed using two site, solid-phase, flash chemiluminescence procedures while small molecules utilize competitive assays. In all cases, separation is effected magnetically using paramagnetic particles. The Analyzer will read the bar codes of all samples in the carousel, query an assay plan server to ascertain which analytes need to be run, verify that the proper reagents are on-board, and run the determinations automatically. Results will be analyzed by the automated assay supervisor and copies transferred by electronic mail to the laboratory manager. Assessments will be made with reference to concomitant QC results. If all is in order, the results will then be transferred both to the Electronic Plan Server and to the Coordinating Center where reasonability and other checks are made. In most cases, assays for all analytes will be completed on the day of sample thawing. Insulated shipping containers will be re-furnished and returned to the sites by second day service. Occasionally, these return shipments will be used to provide specimen collection packets needed by sites.

6.7.3 Assays to be Performed at Medical Research Laboratories

Lipid Profile, Lipoproteins

Total Cholesterol, Triglyceride, HDL Cholesterol, Calculated LDL Cholesterol

Lipoproteins Lp(a) and Lp(A1)

Fibrinogen, Factor VIIc, PAI-1, TPA Antigen

Glucose and Insulin

Bone Markers (serum and urine)

6.7.4 Assays and QC Procedures at Medical Research Laboratories

The analytical methods and quality assurance program (QAP) that will be utilized for the Study of Women's Health Across the Nation will be identical to those used for the Women's Health Initiative (WHI).

Briefly, the methods including calibration and quality assurance will be maintained throughout the duration of this four-year (4) study. The methodology for these analytes has been operational for some time and is well standardized. No changes are anticipated. In addition to the routine internal and external quality assurance programs, monitoring for analytical stability will utilize a system devised for the Women's Health Initiative. The Women's Health Initiative will run concurrently with the proposed study. This specifically devised quality assurance program involves specially created serum and plasma pools drawn from a single donor. Each pool has been aliquotted into 180 cryovials and frozen at -70°C . These samples are stored at Ogden Bioscience in Maryland, along with samples from participants in WHI. For each analyte, two (2) pools have been formulated. Each pool for each analyte will be analyzed on a monthly basis for the next 12 years. This will be carried out in a blinded fashion with the pool sample indistinguishable from study participant samples. As the measurements in this study are the same as those for the WHI, all analytes anticipated to be measured for the proposed study will also be closely monitored.

SPECIMEN HANDLING SPECIFICATIONS

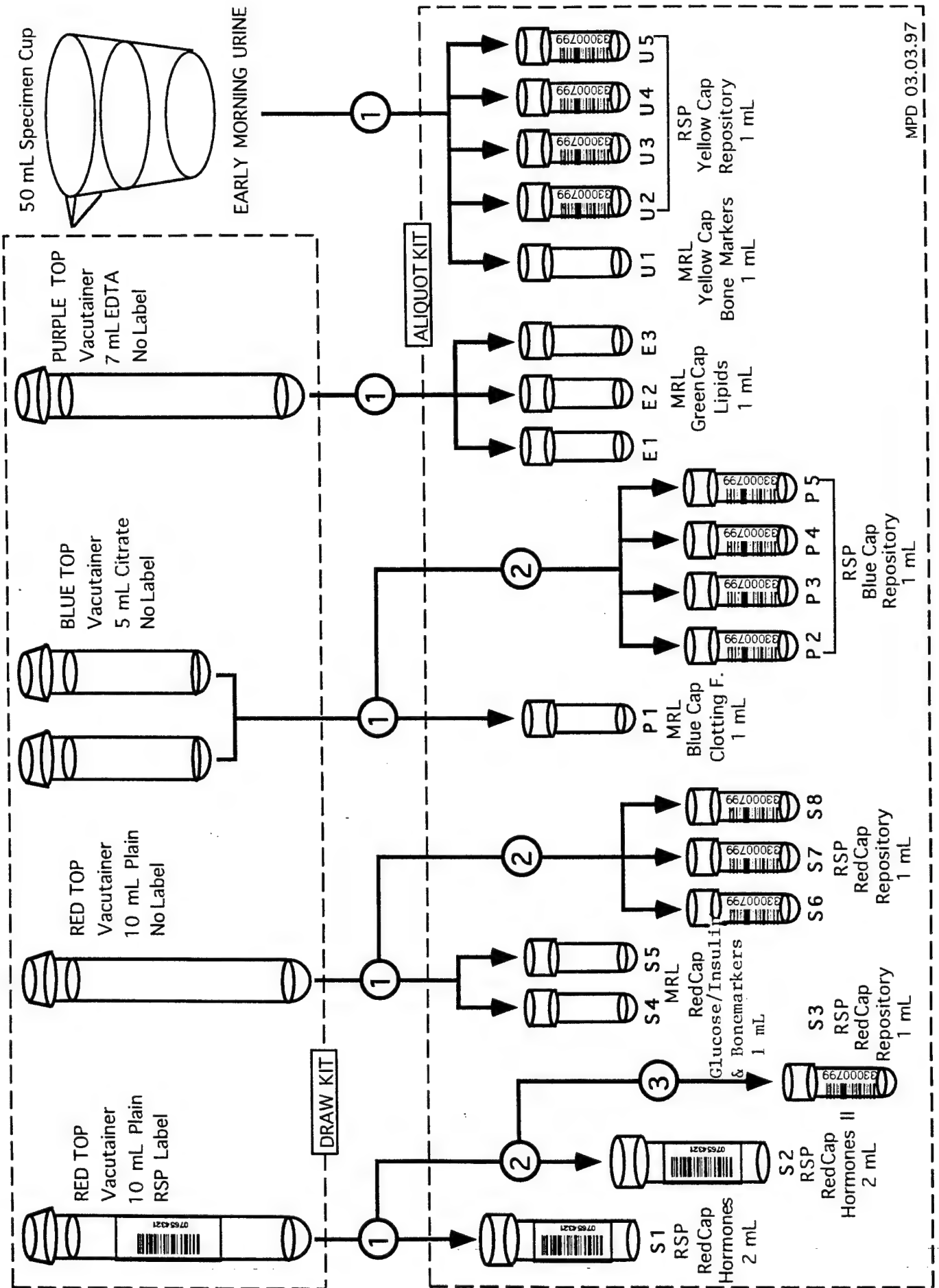
DRAW (in order) Volume/Tube	PROCESSING Timing, Spinning, etc.	ALIUQUOTTING Volume, Tube	STORAGE	SHIPPING Lab, Frequency	ASSAYS
#1 10 mL Red Top (Serum)	Invert 8-10 times 30-60* at room temp. Refrig. 4°C 30-60 min. * Centrifuge 4°C ≈ 1300 g (approx. 2000 rpm) 20 min.	2 mL in a 5 mL red tube 2 mL in a 5 mL red tube 1 mL in a 2 mL red tube	Put tubes in RSP Shipment #1 and #2 bags and freeze upright in ≤ -20°C freezer < 30 days	RSP-1 Monthly Dry Ice RSP-2 Monthly Dry Ice ** RSP-1 Monthly Dry Ice	Endo. Hormones Back-up Endo. Hormones Repository Serum
#2 10 mL Red Top (Serum)	Invert 8-10 times 30-60* mins. at room temp. Refrig. 4°C 30-60 min. * Centrifuge 4°C ≈ 1300 g (approx. 2000 rpm) 20 min.	1 mL in a 3.5 mL red tube 1 mL in a 3.5 mL red tube 1 mL in a 2 mL red tube 1 mL in a 2 mL red tube 1 mL in a 2 mL red tube	Put the two 3.5 mL tubes in the MRL shipment bag, put the three 2.0 mL tubes in RSP Shipment bags (#1 and #2) and freeze ALL BAGS upright in ≤ -20°C freezer < 30 days	MRL Monthly Dry Ice MRL Monthly Dry Ice RSP-1 Monthly Dry Ice RSP-2 Monthly Dry Ice RSP-2 Monthly Dry Ice	Glucose; Insulin Bone markers Repository Serum Repository Serum Repository Serum
#3 5 mL Blue Top (Citrate)	Invert 8-10 times Place in wet or blue ice (or refrigerate) for up to 2 hrs Centrifuge 4°C ≈ 1300 g (approx. 2000 rpm) 20 min	1 mL in a 3.5 mL blue tube 1 mL in a 2 mL blue tube 1 mL in a 2 mL blue tube	Put the 3.5 mL tube in the MRL shipment bag, put the other tubes in RSP Shipment bags (#1 and #2) and freeze BOTH BAGS immediately at ≤ -20°C. MUST be frozen at least overnight.	MRL Monthly Dry Ice RSP-1 Monthly Dry Ice RSP-2 Monthly Dry Ice	Clotting Factors Repository(plasma) Repository (plasma)
#4 7 mL Lavender Top (EDTA)	Invert 8-10 times Place in wet or blue ice (or refrigerate) for up to 2 hrs Centrifuge 4°C ≈ 1300 g (approx. 2000 rpm) 20 min	1 mL in a 3.5 mL green tube 1 mL in a 3.5 mL green tube 1 mL in a 3.5 mL green tube	Put the three 3.5 mL tubes in the MRL bag and freeze upright in ≤ 20°C freezer < 30 days	MRL Monthly Dry Ice MRL Monthly Dry Ice MRL Monthly Dry Ice	Lipids (EDTA) CV Markers CV Markers
#5 5 mL Blue Top (Citrate)	Invert 8-10 times Place in wet or blue ice (or refrigerate) for up to 2 hrs Centrifuge 4°C ≈ 1300 g (approx. 2000 rpm) 20 min	1 mL in a 2 mL blue tube 1 mL in a 2 mL blue tube	Put the tubes in RSP Shipment bags (#1 and #2) and freeze BOTH BAGS immediately at ≤ - 20°C. MUST be frozen at least overnight.	RSP-1 Monthly Dry Ice RSP-2 Monthly Dry Ice	Repository (plasma) Repository (plasma)
Urine Sample	Protect sample from direct sun rays. Immediate aliquotting and freezing preferred. May refrigerate 4°C upon receiving OR place in cooler on blue ice <4hrs.	1 mL in a 3.5mL yellow tube 1 mL in a 2 mL yellow tube 1 mL in a 2 mL yellow tube 1 mL in a 2 mL yellow tube 1 mL in a 2 mL yellow tube	Put the 3.5 mL tube in the MRL bag. Put the first two 2.0 mL vials into the RSP Shipment #1 bag and the second two vials into the RSP Shipment #2 bag and freeze ALL BAGS immediately ≤ -20°C	MRL Monthly Dry Ice RSP-1 Monthly Dry Ice RSP-1 Monthly Dry Ice RSP-2 Monthly Dry Ice RSP-2 Monthly Dry Ice	Urine Bone Markers Repository Repository Repository Repository

* Preferred that red tops sit at room temp for 60 minutes, then refrigerated for 60 minutes before centrifuging.

** RSP-2 identifies a tube to be included in a separate shipment from RSP-1 tubes (to avoid total loss of repository aliquots).

Figure 6.2

Specimen Processing 'Follow Up' Module



Study of Women's Health Across the Nation Specimen Collection Record

Version 02/01/97

SECTION A. GENERAL INFORMATION

A1. SUBJECT ID:

AFFIX ID LABEL HERE

A5. SUBJECTS DOB

M M / D D / Y Y Y Y

A2. SWAN STUDY VISIT #

A6. SUBJECT'S POSITION WHILE BLOOD DRAWN (Circle One)

1. Sitting 2. Lying Down

A3. FORM VERSION: 02/01/1997

A7. WAS BUTTERFLY NEEDLE USED

1. No (B1A) 2. Yes (A7A)

A4. DATE FORM COMPLETED

M M / D D / Y Y Y Y

A7A. WHAT GAUGE? G

SECTION B.

Draw/Collection	B1. Blood Draw Completed	B2. Collection Date (✓ Box if same as line above)	B3. Collection Time (✓ Box if same as line above)	B4. Initials of Phlebotomist (✓ Box if same as line above)	B5. Refrig. before Spinning	B6. Start of Spln Time (✓ Box if same as line above)	B7. Time aliquots placed in freezer or refrigerator after spinning (Temperature of freezer) (✓ Box if same as line above)	B8. Initials of Laboratorian (✓ Box if same as line above)
10 mL Red Top #1	Y N	___/___/___	1. AM : : 2. PM		Y N	1. AM : : 2. PM	1. AM °C : : 2. PM	
10 mL Red Top #2	Y N	___/___/___	1. AM : : 2. PM		Y N	1. AM : : 2. PM	1. AM °C : : 2. PM	
5 mL Blue Top #1	Y N	___/___/___	1. AM : : 2. PM		Y N	1. AM : : 2. PM	1. AM °C : : 2. PM	
7 mL Purple Top	Y N	___/___/___	1. AM : : 2. PM		Y N	1. AM : : 2. PM	1. AM °C : : 2. PM	
5 mL Blue Top #2	Y N	___/___/___	1. AM : : 2. PM		Y N	1. AM : : 2. PM	1. AM °C : : 2. PM	
50 mL Urine Specimen Cup	Y N	___/___/___	1. AM : : 2. PM	N/A	N/A	N/A	1. AM °C : : 2. PM	

B9. NOTES:


Figure 6.4a

Figure 6.4b


C1. 1 x . . nL Red Top <input type="checkbox"/> Check if All Serum Obtained Aliquots: 2 x 2 mL Aliquots 1 x 1 mL Aliquots As Per Protocol	C2. 1 x 10 mL Red Top <input type="checkbox"/> Check if All Serum Obtained Aliquots: 5 x 1 mL Aliquots As Per Protocol	C3. 1 x 5 mL Blue Top <input type="checkbox"/> Check if All Citrated Plasma Obtained Aliquots: 3 x 1 mL Aliquots As Per Protocol	C4. 1 x 5 mL Blue Top <input type="checkbox"/> Check if All Citrated Plasma Obtained Aliquots: 2 x 1 mL Aliquots As Per Protocol	C5. 1 x 7 mL Purple Top <input type="checkbox"/> Check if All EDTA Plasma Obtained Aliquots: 3 x 1 mL Aliquots As Per Protocol	C6. 1 x . . . Urine <input type="checkbox"/> Check if All Urine Obtained Aliquots: 5 x 1 mL Aliquots As Per Protocol
S1 2 ml in 5ml vial Serum Endo 99000000 --- RSP 1	S4 1 ml in 3.5 ml vial --- MRL	P1 1 ml in 3.5 ml vial --- MRL	P4 1 ml in 2 ml vial Plasma Repository 99000000 --- RSP 1	E1 1 ml in 3.5 ml vial --- MRL	U1 1 ml in 3.5 ml vial --- MRL
S2 2 ml in 5ml vial Serum Endo 99000000 --- RSP 2	S5 1 ml in 3.5 ml vial --- MRL	P2 1 ml in 2 ml vial Plasma Repository 99000000 --- RSP 1	P5 1 ml in 2 ml vial Plasma Repository 99000000 --- RSP 2	E2 1 ml in 3.5 ml vial --- MRL	U2 1 ml in 2 ml vial Urine Repository 99000000 --- RSP 1
S3 1 ml in 2 ml vial Serum Repository 99000000 --- RSP 1	S6 1 ml in 2 ml vial Serum Repository 99000000 --- RSP 1	P3 1 ml in 2 ml vial Plasma Repository 99000000 --- RSP 2	<div style="border: 1px solid black; padding: 5px; text-align: center;">000</div>	E3 1 ml in 3.5 ml vial --- MRL	U3 1 ml in 2 ml vial Urine Repository 99000000 --- RSP 1
S7 1 ml in 2 ml vial Serum Repository 99000000 --- RSP 2	S8 1 ml in 2 ml vial Serum Repository 99000000 --- RSP 2	<div style="border: 1px solid black; padding: 5px;"> NOTES Aliquot _____ Problem _____ _____ _____ _____ _____ _____ </div>			
- MODULE - 99000000	- DRAW KIT - 99000000	- ALIQUOT KIT - 99000000	- VACUTAINER - 99000000	<div style="border: 1px solid black; padding: 5px; text-align: center;"> Specimen Collection Log version 02.01.97 </div>	

Complete Corresponding Volumes for Each Expected Aliquot. If any Aliquots Not Obtained or Partially Filled for any Specimen Type Round Volumes to Nearest 1/4 mL i.e. 0.50 mL, 0.75 mL, or 0.00 if not obtained


DRAW TUBE

13-0161 DMT: ---
 Year 1
 Visit 2 / / /
 SWAN mm dd yy
 ** Glucose/Insulin **

 GUA00ACC


TRANSFER VIAL


13-0161 DMT: ---
 Year 1
 Visit 2 / / /
 SWAN mm dd yy
 ** Glucose/Insulin **

 GUA00ACC


SHIPPING LIST

13-0161 DMT: ---
 Year 1
 Visit 2 / / /
 SWAN mm dd yy
 ** Glucose/Insulin **

 GUA00ACC


4.5 ml
 citrate


13-0161 DMT: ---
 Year 1
 Visit 2 / / /
 SWAN mm dd yy
 ***** Coagulation *****

 GUA00ACC


13-0161 DMT: ---
 Year 1
 Visit 2 / / /
 SWAN mm dd yy
 ***** Coagulation *****

 GUA00ACC

13-0161 DMT: ---
 Year 1
 Visit 2 / / /
 SWAN mm dd yy
 ***** Coagulation *****

 GUA00ACC


Urine


13-0161 DMT: ---
 Year 1
 Visit 2 / / /
 SWAN mm dd yy
 Urine Bone Markers

 GUA00ACC


13-0161 DMT: ---
 Year 1
 Visit 2 / / /
 SWAN mm dd yy
 Urine Bone Markers

 GUA00ACC


13-0161 DMT: ---
 Year 1
 Visit 2 / / /
 SWAN mm dd yy
 Urine Bone Markers

 GUA00ACC


7.0 ml
 EDTA


13-0161 DMT: ---
 Year 1
 Visit 2 / / /
 SWAN mm dd yy
 ***** Lipid 1 *****

 GUA00ACC

13-0161 DMT: ---
 Year 1
 Visit 2 / / /
 SWAN mm dd yy
 ***** Lipid 1 *****

 GUA00ACC

13-0161 DMT: ---
 Year 1
 Visit 2 / / /
 SWAN mm dd yy
 ***** Lipid 1 *****

 GUA00ACC

13-0161 DMT: ---
 Year 1
 Visit 2 / / /
 SWAN mm dd yy
 ***** Lipid 2 *****

 GUA00ACC

13-0161 DMT: ---
 Year 1
 Visit 2 / / /
 SWAN mm dd yy
 ***** Lipid 2 *****

 GUA00ACC

13-0161 DMT: ---
 Year 1
 Visit 2 / / /
 SWAN mm dd yy
 ***** Lipid 3 *****

 GUA00ACC


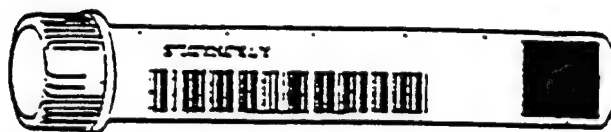
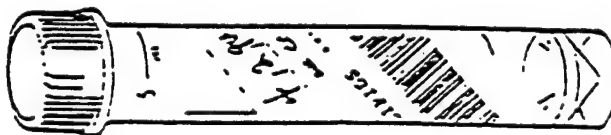
13-0161 DMT: ---
 Year 1
 Visit 2 / / /
 SWAN mm dd yy
 ***** Lipid 3 *****

 GUA00ACC

Figure 6.7

CORRECT PLACEMENT OF BARCODE LABEL



INCORRECT PLACEMENT OF BARCODE LABEL



Shipping Record to RSP

Complete this form for each shipment to RSP. The information is to identify each bag of samples included in a single shipment. Attach a copy of the Airborne Express airbill to the completed sheet. Optionally attach a printed copy of the e-mail message acknowledging receipt of the samples at RSP. File the attached sheets locally as part of the site records.

Date shipped: _____

Initials, person shipping: _____

Airbill number (upper right on airbill): _____

First shipment of samples to RSP:

- ☐ one (1) red cap tube with ~2 mL Serum (endocrine hormones);
- ☐ two (2) red cap vials with ~1 mL Serum (repository);
- ☐ two (2) blue cap vials with ~1 mL Citrate plasma (repository), and
- ☐ two (2) yellow cap vials with ~1 mL Urine (repository).

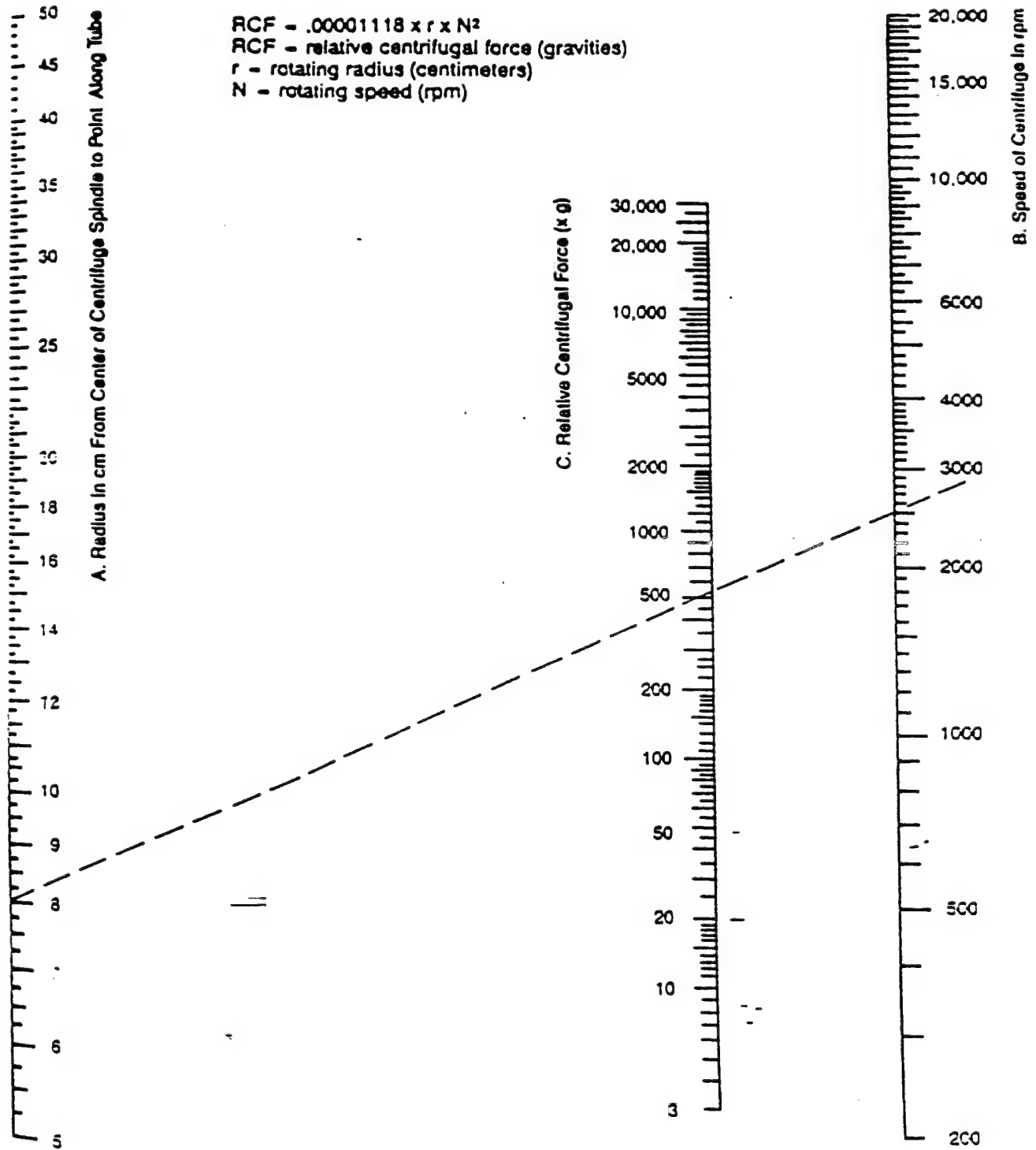
Second shipment of samples from same person to RSP:

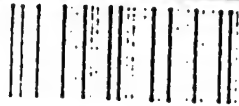
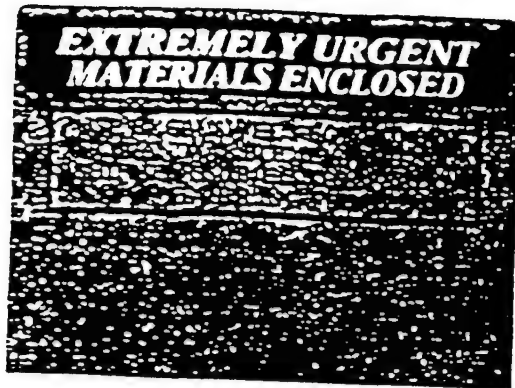
- ☐ one (1) red cap tube with ~2 mL Serum (backup for endocrine hormones);
- ☐ two (2) red cap vials with ~1 mL Serum (repository);
- ☐ two (2) blue cap vials with ~1 mL Citrate plasma (repository), and
- ☐ two (2) yellow cap vials with ~1 mL Urine (repository).

#	Subject Initials	Date Collected	3 Digit Code	Shipment 1 or 2
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
11				
12				
13				
14				
15				
16				
17				
18				
19				
20				

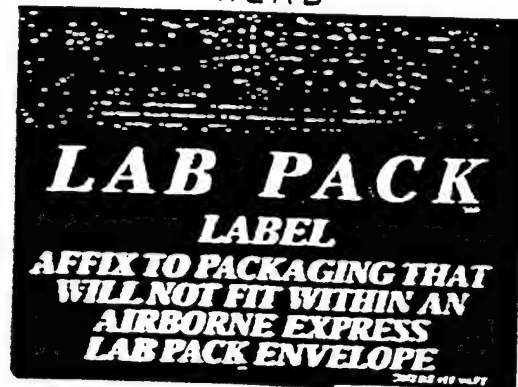
#	Subject Initials	Date Collected	3 Digit Code	Shipment 1 or 2
21				
22				
23				
24				
25				
26				
27				
28				
29				
30				
31				
32				
33				
34				
35				
36				
37				
38				
39				
40				

Nomogram for Calculating RCF





HLAB



Shipper's Declaration not Required

Dry ice amount must be
in kilograms

2 lbs = 1 kg

5 lbs = 2.3 kg

Airbills must have the following:

1. Dangerous goods, shippers
declaration not required
2. Dry ice: 9; UN1845
3. 1 x _____ Kg 904111
(w1)



Dry Ice
_____ kg

Shipper's Name and Address

UN 1845

Consignee Name and Address
Medical Research Laboratories
2 Tessenear Drive
Highland Heights KY 41076

FOR SHIPMENTS WITHIN U.S. ONLY

[illegible]

Appendix B**SWAN Specimen Collection and Processing Forms**

Study of Women's Health Across the Nation Specimen Collection Record

Version 02/01/97

SECTION A. GENERAL INFORMATION

A1. SUBJECT ID:

AFFIX ID LABEL HERE

A5. SUBJECTS DOB

M M / D D / Y Y Y Y

A2. SWAN STUDY VISIT #

A6. SUBJECT'S POSITION WHILE BLOOD DRAWN (Circle One)

 1. Sitting
2. Lying Down

A3. FORM VERSION: 02/01/1997

A7. WAS BUTTERFLY NEEDLE USED

1. No (B1A) 2. Yes (A7A)

A4. DATE FORM COMPLETED

M M / D D / Y Y Y Y

A7A. WHAT GAUGE? ____ G

SECTION B.













	B1.	B2.	B3.	B4.	B5.	B6.	B7.	B8.
	Blood Draw Completed	Collection Date (✓ Box if same as line above)	Collection Time (✓ Box if same as line above)	Initials of Phlebotomist (✓ Box if same as line above)	Refrig. before Spinning	Start of Spin Time (✓ Box if same as line above)	Time aliquots placed in freezer or refrigerator after spinning (Temperature of freezer) (✓ Box if same as line above)	Initials of Laboratorian (✓ Box if same as line above)
10 mL Red Top #1	Y N	____/____/____	____:____:____ 1. AM 2. PM	____	Y N	____:____:____ 1. AM 2. PM	____:____:____ 1. AM 2. PM	____
10 mL Red Top #2	Y N	____/____/____	____:____:____ 1. AM 2. PM	____	Y N	____:____:____ 1. AM 2. PM	____:____:____ 1. AM 2. PM	____
5 mL Blue Top #1	Y N	____/____/____	____:____:____ 1. AM 2. PM	____	Y N	____:____:____ 1. AM 2. PM	____:____:____ 1. AM 2. PM	____
7 mL Purple Top	Y N	____/____/____	____:____:____ 1. AM 2. PM	____	Y N	____:____:____ 1. AM 2. PM	____:____:____ 1. AM 2. PM	____
5 mL Blue Top #2	Y N	____/____/____	____:____:____ 1. AM 2. PM	____	Y N	____:____:____ 1. AM 2. PM	____:____:____ 1. AM 2. PM	____
50 mL Urine Specimen Cup	Y N	____/____/____	____:____:____ 1. AM 2. PM	N/A	N/A	N/A	____:____:____ 1. AM 2. PM	____

B9. NOTES:

Figure 6.4b

C1. 1 x . . mL Red Top <input type="checkbox"/> Check if All Serum Obtained Aliquots: 2 x 2 mL Aliquots 1 x 1 mL Aliquots As Per Protocol	C2. 1 x 10 mL Red Top <input type="checkbox"/> Check if All Serum Obtained Aliquots: 5 x 1 mL Aliquots As Per Protocol	C3. 1 x 5 mL Blue Top <input type="checkbox"/> Check if All Citrated Plasma Obtained Aliquots: 3 x 1 mL Aliquots As Per Protocol	C4. 1 x 5 mL Blue Top <input type="checkbox"/> Check if All Citrated Plasma Obtained Aliquots: 2 x 1 mL Aliquots As Per Protocol	C5. 1 x 7 mL Purple Top <input type="checkbox"/> Check if All EDTA Plasma Obtained Aliquots: 3 x 1 mL Aliquots As Per Protocol	C6. 1 x . . mL Urine <input type="checkbox"/> Check if All Urine Obtained Aliquots: 5 x 1 mL Aliquots As Per Protocol
-----------------------------------------------------------------------------------------------------------------------------------------------------------------	-------------------------------------------------------------------------------------------------------------------------------------------	-----------------------------------------------------------------------------------------------------------------------------------------------------	-----------------------------------------------------------------------------------------------------------------------------------------------------	---------------------------------------------------------------------------------------------------------------------------------------------------	------------------------------------------------------------------------------------------------------------------------------------------

Complete Corresponding Volumes for Each Expected Aliquot. If any Aliquots Not Obtained or Partially Filled for any Specimen Type Round Volumes to Nearest 1/4 mL i.e. 0.50 mL, 0.75 mL, or 0.00 if not obtained

S1 2 ml in 5ml vial Serum Endo  990000000 --- RSP 1	S2 2 ml in 5ml vial Serum Endo  990000000 --- RSP 2	S3 1 ml in 2 ml vial Serum Repository  990000000 --- RSP 1	S4 1 ml in 3.5 ml vial --- MRL	S5 1 ml in 3.5 ml vial --- MRL	S6 1 ml in 2 ml vial Serum Repository  990000000 --- RSP 1
<div style="border: 1px solid black; padding: 10px; width: fit-content; margin: 0 auto;">000</div>			P1 1 ml in 3.5 ml vial --- MRL	P2 1 ml in 2 ml vial Plasma Repository  990000000 --- RSP 1	P3 1 ml in 2 ml vial Plasma Repository  990000000 --- RSP 2
<div style="border: 1px solid black; padding: 10px; width: fit-content; margin: 0 auto;">000</div>			P4 1 ml in 2 ml vial Plasma Repository  990000000 --- RSP 1	P5 1 ml in 2 ml vial Plasma Repository  990000000 --- RSP 2	E1 1 ml in 3.5 ml vial --- MRL
<div style="border: 1px solid black; padding: 10px; width: fit-content; margin: 0 auto;">000</div>			E2 1 ml in 3.5 ml vial --- MRL	E3 1 ml in 3.5 ml vial --- MRL	U1 1 ml in 3.5 ml vial --- MRL
<div style="border: 1px solid black; padding: 10px; width: fit-content; margin: 0 auto;">000</div>			U2 1 ml in 2 ml vial Urine Repository  990000000 --- RSP 1	U3 1 ml in 2 ml vial Urine Repository  990000000 --- RSP 1	U4 1 ml in 2 ml vial Urine Repository  990000000 --- RSP 2
<div style="border: 1px solid black; padding: 10px; width: fit-content; margin: 0 auto;">000</div>			U5 1 ml in 2 ml vial Urine Repository  990000000 --- RSP 2		

NOTES	Problem	
Aliquot		

Specimen Collection Log

version 02.01.97